(26) J. R. Piper, C. R. Stringfellow, Jr., and T. P. Johnston, *J. Med. Chem.*, **9**, 911 (1966).
(27) R. Paul, R. P. Williams, and E. Cohen, *J. Org. Chem.*, **40**, 1653 (1975).
(28) J. R. Piper and T. P. Johnston, *J. Org. Chem.*, **32**, 1261 (1967).
(29) H. Strache, *Ber. Dtsch. Chem. Ges.*, 2358 (1888).
(30) R. Adams and T. L. Cairns, *J. Am. Chem. Soc.*, **61**, 2464 (1939).
(31) J. D. Roberts and R. H. Mazur, *J. Am. Chem. Soc.*, **73**, 2509 (1951).
(32) A. Hassner and C. Heathcock, *J. Org. Chem.*, **30**, 1748 (1965).
(33) L. Macholan, *Collect. Czech. Chem. Commun.*, **30**, 2074 (1965).
(34) W. Kirmse and D. Grassmann, *Chem. Ber.*, **99**, 1746 (1966).
(35) S. Gabriel and H. Ohle, *Ber. Dtsh. Chem. Ges.*, **50**, 819 (1917).
(36) D. H. Ball, J. M. Williams, and L. Long, Jr., *J. Org. Chem.*, **28**, 1589 (1963).
(37) Prepared in DMF like *N*-(4-bromobutyl)phthalimide [J. R. Piper and T. P. Johnston, *J. Org. Chem.*, **33**, 636 (1968)] and recrystallized from hexane, yield 57%.
(38) S. Takagi and K. Hayashi, *Chem. Pharm. Bull.*, **7**, 183 (1959).
(39) E. E. van Tamalen and E. E. Smissman, *J. Am. Chem. Soc.*, **75**, 2031 (1953).
(40) S. Akerfeldt, *Acta Chem. Scand.*, **16**, 1897 (1962).
(41) D. L. Klayman and E. S. Copeland, *Drug Des.*, **6**, 81 (1975).
(42) P. Karrer and M. Ehrenstein, *Helv. Chim. Acta*, **9**, 323 (1926).

Studies on Biologically Active Nucleosides and Nucleotides. 5. Synthesis and Antitumor Activity of Some 2,2′-Anhydro-1-(3′,5′-di-*O*-acyl-β-D-arabinofuranosyl)cytosine Salts and 2,2′-Anhydro-1-(3′-*O*-acyl-β-D-arabinofuranosyl)cytosine 5′-Phosphates

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A series of 3′,5′-diesters of a 2,2′-anhydro-1-(β-D-arabinofuranosyl)cytosine salt bearing functional substituents on the ester side chains (**4–16**) have been synthesized. The synthesis of these diesters involved the reaction between cytidine and the corresponding acid anhydride or acid chloride in the presence of boron trifluoride etherate. Similar reaction of bis(cytidine 5′-)suberate (**21**) with pivaloyl chloride gave bis[2,2′-anhydro-1-(3′-*O*-pivaloyl-β-D-arabinofuranosyl)cytosine 5′-]suberate dihydrochloride (**22**). The reaction could also be extended to a one-step synthesis of 3′-esters of 2,2′-anhydro-1-(β-D-arabinofuranosyl)cytosine 5′-phosphate (**24**) from 5′-cytidylic acid. These compounds have been examined for antitumor activity against L1210 leukemia in BDF_1 mice. The diesters with a long-chain carboxylic acid (**4c**, **7c**, **12**, and **24d**) showed high activity when administered intraperitoneally. The compound **24d** exhibited moderate activity when administered orally.

2,2′-Anhydro-1-(β-D-arabinofuranosyl)cytosine (anhydro-*ara*-C) hydrochloride, a depot form of 1-(β-D-arabinofuranosyl)cytosine (*ara*-C),[1] is a more effective and less toxic antitumor agent than is *ara*-C itself.[2] A number of analogues of anhydro-*ara*-C with modification in the base and/or sugar portions have been prepared in an attempt to find more potent antitumor agents. Kanai et al.[3] reported the synthesis of 2,2′-anhydro-1-(β-D-arabinofuranosyl)cytosine 5′-phosphate (anhydro-*ara*-C 5′-phosphate) by the reaction of 5′-cytidylic acid (**23**) with partially hydrolyzed phosphorus oxychloride in 46% yield. This compound has shown marked activity against L1210 leukemia in mice.[4] Very recently, Moffatt and co-workers[5] have synthesized 3′,5′-di-*O*-acyl derivatives of anhydro-*ara*-C with a homologous series of aliphatic saturated or unsaturated carboxylic acids by the acylation of anhydro-*ara*-C hydrochloride. They have also shown that these diesters have activity against L1210 leukemia in mice when administered intraperitoneally (ip). The degree of the activity was strongly influenced by the chain length; high activity was shown by the compound containing C_{12}–C_{14} saturated acyl groups and C_{18}–C_{22} unsaturated acyl groups.

In a previous study in this series, we have developed a simple and efficient method for the preparation of the diesters by the reaction of cytidine with carboxylic acid anhydrides or carboxylic acid chlorides in the presence of boron trifluoride etherate.[6] The mechanism for the formation of the diesters could be explained via the opening of the 2′,3′-*O*-acyloxonium ion intermediate **3** by C_2-carbonyl oxygen (Scheme I). Using this procedure, we have prepared a series of the diesters with simple aliphatic and aromatic carboxylic acids and have tested[7] them for antileukemic (L1210) activity in mice. Among the compounds tested, 2,2′-anhydro-1-(3′,5′-di-*O*-palmitoyl-β-D-arabinofuranosyl)cytosine hydrochloride was found to be highly active at 100 (mg/kg)/day (5 days), ip (see Table II). However, the therapeutic index could not be improved compared to anhydro-*ara*-C because of the increased toxicity, and it showed only slight activity when administered orally (po).

On the other hand, it has been shown that some 5′-*O*-acyl-*ara*-C compounds with low water solubility and susceptibility to the enzymatic hydrolysis exhibit high anti-L1210 activity in mice.[8] However, clinical phase 1 trials of such derivatives, 5′-*O*-palmitoyl- and 5′-*O*-benzoyl-*ara*-C, have given discouraging results, suggesting that somewhat increased water solubility would be necessary for the antileukemic activity in man.[9] In view of these facts that the biological activity of the esters of anhydro-*ara*-C and *ara*-C is markedly affected by the precise nature of the acyl group(s), it was of interest to investigate an extensive modification of the acyl groups of the 3′,5′-di-*O*-acyl-anhydro-*ara*-C compounds. We have synthesized a series of the diesters (**4–16**, **22**, and **24**) bearing a variety of functional substituents on the ester side chains. Such modification may affect the water solubility, rate of the enzymatic hydrolysis, and other properties of the diesters and bring about improvement of the therapeutic properties of anhydro-*ara*-C.

Chemistry. The diesters **4–16** synthesized in this study are listed in Table II. These diesters were prepared by the acid chloride and acid anhydride methods. The key intermediates in these methods are functionally substituted carboxylic acid anhydrides (**2a**) and carboxylic acid

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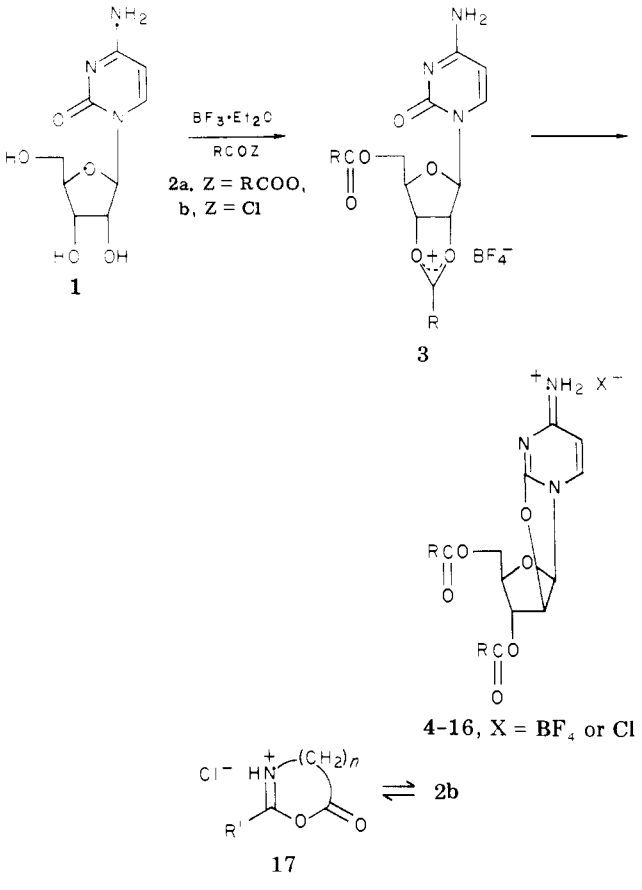
Table I. Characterized Carboxylic Acids, Acid Anhydrides (2a), and Acid Chloride (2b)

structure	formula	anal.	mp or bp (mm), °C	recrystn solvent	yield, %	IR, cm^{-1}
Carboxylic Acids						
3-(heptyloxy)propionic	$C_{10}H_{20}O_3$	C, H	109-111 (0.4)		68	1715
3-(dodecyloxy)propionic	$C_{15}H_{30}O_3$	C, H	55-56	hexane	30	1697
11-(butyrylamino)-undecanoic	$C_{15}H_{29}NO_3$	C, H, N	89-91	H_2O-EtOH	39	1692-1720, 3300
11-(benzoylamino)-undecanoic	$C_{18}H_{27}NO_3$	C, H, N	90-92	*a*	46	1628, 1690, 3303
6-phthalimidohexanoic	$C_{14}H_{15}NO_4$	C, H, N	111-113	*i*-PrOH	91	1698-1720, 1775
11-phthalimido-undecanoic	$C_{19}H_{25}NO_4$	C, H, N	92-93	EtOH	61	1698-1723, 1770
Acid Anhydrides 2a						
11-(butyryloxy)-undecanoic	$C_{30}H_{54}O_5$	C, H	41-42	pentane	68	1740, 1820
3-(dodecyloxy)propionic	$C_{30}H_{58}O_5$	C, H	52-54	Me_2CO	59	1744, 1805
Acid Chloride 2b						
11-(*p*-toluenesulfonyl-amino)undecanoyl	$C_{18}H_{28}NO_3SCl$	C, H, N	81-82	*i*-Pr_2O-pet ether	86	1790, 3260

[a] Purified by chromatography on silicic acid.

Scheme I

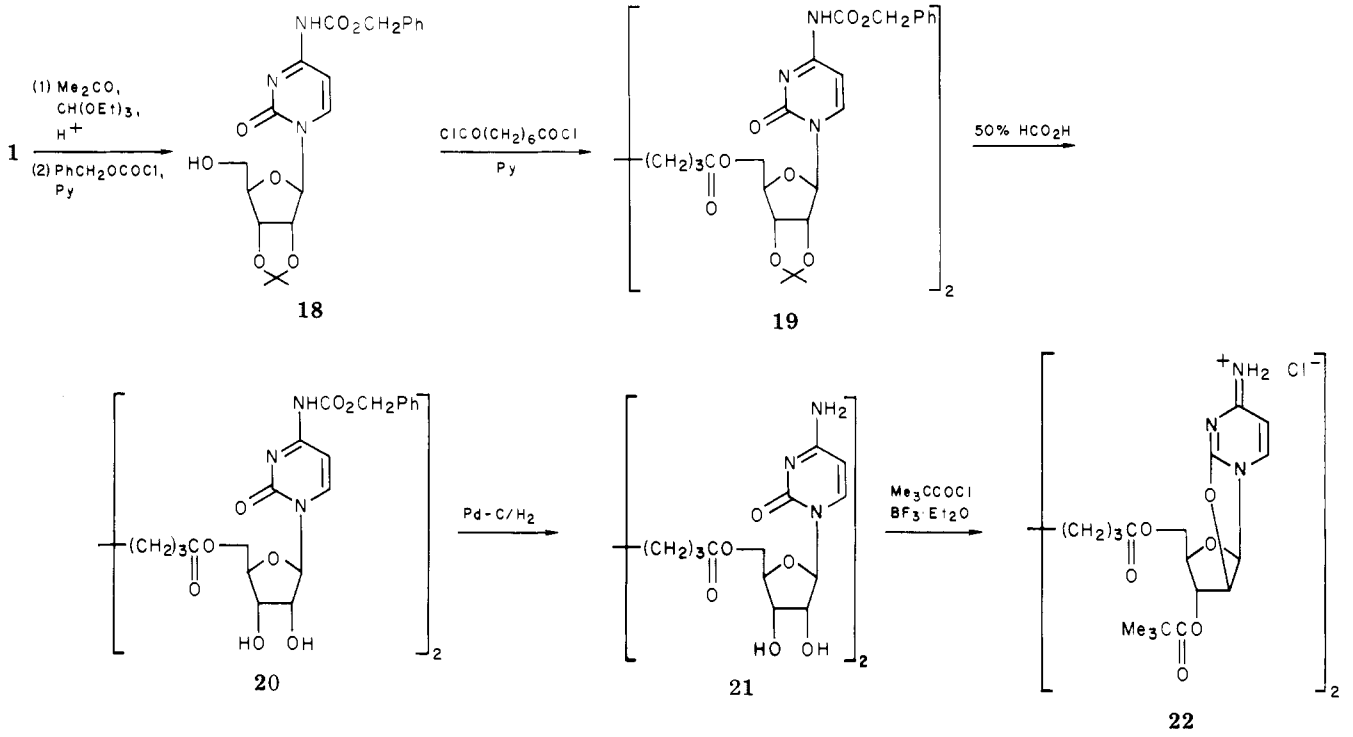


chlorides (**2b**). These carboxylic acids were prepared essentially according to known procedures. Thus, 3-alkoxypropionic acids were obtained by the reaction of β-propiolactone with the appropriate alcohols.[10] ω-Acylamino, ω-tosylamino, and ω-phthalimido acids were prepared by conventional procedures.[11] 11-(Butyryloxy)undecanoic acid was prepared by the reaction of 11-bromoundecanoic acid with sodium butyrate.[12]

Conversion of the carboxylic acids to the corresponding acyl chlorides was carried out by treatment with an excess of thionyl chloride. ω-Tosylamino acid chlorides were prepared by treating the corresponding carboxylic acids with phosphorus pentachloride in ether.[13] The infrared spectra of all of the crude products from ω-acylamino acids did not exhibit the expected NH-stretching absorption but showed bands at 2250–2450 and near 1800 cm^{-1}, attributable to NH^+ and carbonyl absorptions. In contrast, the spectra of the corresponding ω-tosylamino acid chlorides exhibited a strong NH absorption near 3300 cm^{-1}. These results indicate that the products exist in an azlactone hydrochloride form (**17**) but not in the acyl chloride form **2b**.[14] A marked enhancement of the carbonyl frequency in azlactones has been reported.[15] In a number of cases, purified acyl chlorides were used in the next step, while in some cases the crude products, which gave satisfactory infrared spectra, were used directly. 3-Alkoxypropionic anhydrides were prepared by the condensation of the corresponding acyl chlorides with the sodium salt of the carboxylic acids.[16] In the case of 3-(dodecyloxy)propionic anhydride, it was possible to obtain the pure anhydride by crystallization of the crude product, while on other occasions the crude anhydrides were used in the next step without purification because they underwent partial decomposition upon attempted distillations. 11-(Butyryloxy)undecanoic anhydride was obtained by dehydration of the corresponding carboxylic acid with dicyclohexylcarbodiimide. The physical properties of the purified carboxylic acids, acid anhydrides, and acid chloride are given in Table I.

The reaction between cytidine (**1**) and acid anhydrides **2a** or acid chlorides **2b** was conducted under the conditions similar to those reported previously.[6] Thus, an excess (3 molar-equiv) of **2a** or **2b** was added to a boiling solution of cytidine and boron trifluoride etherate (3 molar-equiv) in acetonitrile (roughly 7 mL/mmol of cytidine). Usually, the reaction was essentially complete within 15 min as judged by TLC and UV analyses. In the case of halogen-substituted benzoyl chlorides, particularly *m*-chlorobenzoyl chloride, the reaction was slow and required a longer refluxing time (1–12 h). Isolation of the pure diester hydrotetrafluoroborates **4-16** (X = BF_4) was achieved either by direct crystallization (purification method A) or by chromatography on silicic acid (purification method B). In a number of cases, the diester hydrotetrafluoroborates which failed to crystallize were converted to the crystalline hydrochlorides by passing them through a column of anion-exchange resin (Cl^- form). From Table II it can be seen that, in general, the yields of the diesters obtained by the purification method B are low. This is largely due to the partial decomposition of the diesters during chromatography on silicic acid. The

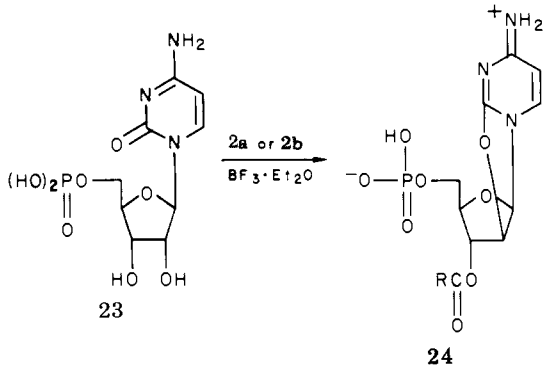
Scheme II



lability of anhydro-*ara*-C compounds toward active adsorbants has been noted.[17] The reaction using 3-(2-furyl)acryloyl chloride **2b** (R = $C_4H_3OCH{=}CH$) was accompanied by severe discoloration, and the desired product **16** (R = $C_4H_3OCH{=}CH$, X = Cl) could only be obtained in 13% yield. Under these conditions, polymerization of the acyl chloride **2b** (R = $C_4H_3OCH{=}CH$) appears to occur. The reaction of 3-ethoxypropionyl chloride **2b** [R = $C_2H_5O(CH_2)_2$] gave a complex mixture that could not be resolved to give the expected bis(3-ethoxypropionyl) ester **4a** [R = $C_2H_5O(CH_2)_2$]. This failure was predominantly due to partial cleavage of the ether linkage by chloride attack on **2b** [R = $C_2H_5O(CH_2)_2$] or on the product **4a**. Thus, examination of the crude product by NMR indicated the presence of a considerable amount of the bis(3-chloropropionyl) ester **10** [R = $Cl(CH_2)_2$] in addition to the desired bis(3-ethoxypropionyl) ester **4a**. The use of a combination of acyl chloride and Lewis acids for the cleavage of ethers has been reported.[18] The preparation of **4a**, however, was achieved by using 3-ethoxypropionic anhydride which generates 3-ethoxypropionate, a more feeble nucleophile than chloride.[19] Accordingly, other bis(3-alkoxypropionyl) derivatives were prepared by the acid anhydride method rather than the acid chloride method. The 3′,5′-di-*O*-acyl derivatives of anhydro-*ara*-C **4–16** prepared as above were characterized by their NMR and UV spectral properties and elemental analyses. Detailed information on the spectral features of 3′,5′-di-*O*-acyl-anhydro-*ara*-C compounds has previously been reported.[5]

As an extension of the above studies, it was decided to prepare bis[2,2′-anhydro-1-(3′-*O*-pivaloyl-β-D-arabinofuranosyl)cytosine 5′-]suberate dihydrochloride (**22**). The synthetic steps leading to the formation of the bis(anhydro-*ara*-C 5′-)suberate derivative **22** are outlined in Scheme II. Treatment of 2′,3′-*O*-isopropylidenecytidine, prepared from cytidine (**1**), with benzyloxycarbonyl chloride in pyridine afforded N^4-[(benzyloxy)carbonyl]-2′,3′-*O*-isopropylidenecytidine (**18**) in an overall yield of 61% from **1**. The structure of **18** was confirmed by NMR spectroscopy, which showed the presence of a benzyloxycarbonyl function at the N^4 position. Reaction of **18** with suberoyl chloride in pyridine yielded the linked nucleoside **19** in 46% yield. Hydrolysis of the isopropylidene function of **19** by 50% formic acid gave the N^4-protected nucleoside **20** in 37% yield. Catalytic hydrogenolysis of **20** gave the deblocked nucleoside **21** as a homogeneous foam that failed to crystallize. The crude **21** was treated with pivaloyl chloride in the presence of boron trifluoride etherate in acetonitrile. Unexpectedly, the reaction led to the formation of a number of products and following treatment with an anion-exchange resin the desired bis(anhydro-*ara*-C 5′-)suberate derivative **22** could be isolated only in 10% yield. The structure of **22** was confirmed by its analytical and spectroscopic properties (see Experimental Section).

Scheme III



The reactions of 5′-cytidylic acid **23** with **2a** or **2b** were carried out under the conditions similar to those used with cytidine itself (Scheme III). In general, the crude products contained less polar byproducts in addition to the desired 3′-*O*-acyl derivatives **24**. Though we have not examined the byproducts, they could be efficiently removed by passing through a column of anion-exchange resin (formate). In this way, the 3′-*O*-acyl derivatives of anhydro-*ara*-C 5′-phosphate, **24**, were prepared in crystalline form in yields of 9 to 32%. The structure of **24** was cleanly

Table II. Acyl Derivatives of the 2,2'-Anhydro-1-(β-D-arabinofuranosyl)cytosine Salt (4-16 and 22) and 2,2'-Anhydro-1-(β-D-arabinofuranosyl)cytosine 5'-Phosphate (24)

no.	structure	formula[a]	meth[b] of prepn	mp, °C	purifn[c] meth	yield, %	UV (MeOH), λ_{max} (ε)	L1210 leukemia, % ILS[d] 6.25 mg/kg, ip	25 mg/kg, ip	100 mg/kg, ip (po)	200 mg/kg, (po)	MTD,[d] mg/kg, ip
					3',5'-Di-*O*-acyl Derivatives of Anhydro-*ara*-C							
4a	3-ethoxypropionyl	$C_{19}H_{27}N_3O_8 \cdot HCl$	AA	168-169	B, DME	21	235 (10 400), 264 (12 000)		15.4	57.5		
4b	3-(heptyloxy)-propionyl	$C_{29}H_{47}N_3O_8 \cdot HCl$	AA	187-189	B, DME	17	235 (8700), 263 (10 600)		28.2			100
4c	3-(dodecyloxy)-propionyl	$C_{39}H_{67}N_3O_8 \cdot HCl$	AA	176-177	B, *i*-PrOH	8	235 (10 900), 263 (12 300)	30.8	118.0	>595.0		500
5a	3-(benzoylamino)-propionyl	$C_{29}H_{29}N_5O_8 \cdot HCl \cdot 1.5H_2O$	AC	117-121	B	10	229 (41 000), 265 (17 000)		2.6	32.1		
5b	11-(butyrylamino)-undecanoyl	$C_{39}H_{65}N_5O_8 \cdot HCl$	AC	160-162	B, DME	5	235 (10 400), 264 (11 800)	20.5	51.3	92.3		500
5c	11-(benzoylamino)-undecanoyl	$C_{45}H_{61}N_5O_8 \cdot HBF_4$	AC	98-103	B, *n*-BuOH	32	230 (31 400), 265 (13 000)	38.5	100.0	>292.3		500
6a	3-(*p*-toluenesulfonyl-amino)propionyl	$C_{24}H_{34}N_5O_{10}S_2 \cdot HBF_4 \cdot 0.2C_4H_9OH$	AC	88-93	B, *n*-BuOH	22	229 (31 000), 264 (10 200)		15.0	47.5		
6b	11-(*p*-toluenesulfonyl-amino)undecanoyl	$C_{45}H_{65}N_5O_{10}S \cdot HBF_4$	AC	73-76	A, *n*-BuOH	46	229 (34 800), 264 (13 900)	12.5	90.0	140.0		500
7a	3-phthalimido-propionyl	$C_{31}H_{25}N_5O_{10} \cdot HBF_4 \cdot 2H_2O$	AC	146-148	A, MeOH	53	219 (51 400) 233 (sh, 23 200) 241 (sh, 16 600), 266 (7700)		2.6	39.7		
7b	6-phthalimido-hexanoyl	$C_{37}H_{37}N_5O_{10} \cdot HBF_4 \cdot 0.5H_2O$	AC	140-141	A, EtOH	50	220 (46 300), 234 (sh, 19 000), 242 (sh, 14 100), 267 (7400)		23.1	60.3		300
7c	11-phthalimido-undecanoyl	$C_{47}H_{57}N_5O_{10} \cdot HBF_4$	AC	87-90	A, EtOH	21	220 (67 900), 234 (sh, 29 900), 242 (sh, 22 600), 267 (10 000)	23.1	76.9	>510.3		500
8a	3-(ethoxycarbonyl)-propionyl	$C_{21}H_{27}N_3O_{10} \cdot HCl$	AC	214-215	A, EtOH	36	235 (8000), 263 (9600)		12.4	35.4 (21.6)		>1000
8b	3-[(undecyloxy)carbonyl]propionyl	$C_{39}H_{63}N_3O_{10} \cdot HCl$	AC	200-202	A, *i*-PrOH	35	236 (9600), 264 (11 300)	15.4	43.6	94.6 (10.8)		750
8c	15-(ethoxycarbonyl)-pentadecanoyl	$C_{45}H_{75}N_3O_{10} \cdot HCl$	AC	166-167	A, EtOH–*i*-Pr_2O	17	235 (9200), 264 (10 300)		10.7	37.3 (6.7)		
9	10-undecenoyl	$C_{31}H_{47}N_3O_6 \cdot HCl$	AC	199-201	A, DME	45	235 (10 100), 264 (11 800)	17.9	51.3	97.5		300
10	3-chloropropionyl	$C_{15}H_{17}Cl_2N_3O_6 \cdot HBF_4$	AC	142-145	A, EtOH	26	235 (10 200), 264 (12 000)			(9.0)	(38.5)	
11	11-bromoundecanoyl	$C_{31}H_{49}Br_2N_3O_6 \cdot HCl \cdot 0.25H_2O$	AC	177-178	A, *i*-PrOH	44	236 (9400), 264 (11 100)	12.8	61.5	145.9		300
12	11-(butylthio)-undecanoyl	$C_{39}H_{67}N_3O_6S_2 \cdot HCl$	AC	169-171	A, DME	27	236 (10 000), 264 (12 000)	26.7	164.0	>624.3 (4.0)	(13.3)	500
13	11-(butyryloxy)-undecanoyl	$C_{39}H_{63}N_3O_{10} \cdot HCl \cdot 0.5H_2O$	AA	157-159	A, AcOEt	43	235 (9600), 264 (11 100)	5.1	66.7	86.7 (13.3)	(33.3)	300
14a	*p*-methylbenzoyl	$C_{25}H_{23}N_3O_6 \cdot HCl$	AC	254-255	A, EtOH	77	242 (44 100), 270 (sh, 13 200)		37.3	81.3 (14.7)	(30.7)	750

14b	*p-tert*-butylbenzoyl	$C_{31}H_{35}N_3O_6 \cdot HCl$	AC	261–262	A, EtOH	44	243 (47 100), 270 (sh, 13 800)	26.7	84.0	116.7 (22.7)	(26.7)	300
14c	*p*-ethoxybenzoyl	$C_{27}H_{27}N_3O_8 \cdot HCl$	AA	253–254	A, MeOH	46	262 (61 200), 273 (sh, 47 800)		33.3	67.5		500
14d	*p*-(octyloxy)benzoyl	$C_{39}H_{53}N_3O_8 \cdot HCl$	AC	224–226	A, MeOH	7	262 (51 400), 273 (sh, 41 400)			12.8		300
14e	*o*-chlorobenzoyl	$C_{23}H_{17}Cl_2N_3O_6 \cdot HCl \cdot 0.5H_2O$	AC	220–222	A, EtOH	51	234 (20 800), 265 (11 600)		25.6	dead	(28.2)	<300
14f	*m*-chlorobenzoyl	$C_{23}H_{17}Cl_2N_3O_6 \cdot HCl$	AC	208–210	A, MeOH	36	234 (27 800), 265 (11 200)		28.2	dead	(38.5)	<300
14g	*p*-chlorobenzoyl	$C_{23}H_{17}Cl_2N_3O_6 \cdot HCl$	AC	245–246	A, EtOH	58	243 (42 700), 270 (sh, 12 000)		31.3	105.1	(21.8)	750
14h	*p*-fluorobenzoyl	$C_{23}H_{17}F_2N_3O_6 \cdot HCl$	AC	214–218	A, EtOH	58	234 (36 200), 263 (13 400)		10.0	47.4	(38.5)	500
15	1-naphthoyl	$C_{31}H_{23}N_3O_6 \cdot HBF_4 \cdot 2H_2O$	AA	189–190	A, $CHCl_3$–MeOH	48	216 (sh, 58 400), 235 (sh, 36 600), 270 (9900), 302 (9300)		35.5	82.1		500
16	3-(2-furyl)acryloyl	$C_{23}H_{19}N_3O_8 \cdot HCl$	AC	254–255	A, EtOH–*i*-PrOH	13	235 (6600), 312 (22 800)		20.0	54.7 (20.0)	(26.7)	300
22	bis(anhydro-*ara*-C 5'-)-suberate derivative	$C_{36}H_{48}N_6O_{12} \cdot 2HCl \cdot 2H_2O$	AC	215–218	A, H_2O	10	235 (20 900), 264 (23 800)		25.0	dead	(41.0)	<300
					3'-*O*-Acyl Derivatives of Anhydro-*ara*-C 5'-Phosphate							
24a	acetyl	$C_{11}H_{14}N_3O_8P \cdot H_2O$[e]	AA	185–195	C, H_2O	31	235 (8200), 264 (9200)		30.7	57.3 (13.3)	(46.9)	>1500
24b	valeryl	$C_{14}H_{20}N_3O_8P$	AA	225	C	31	235 (7000), 264 (7700)	15.8	37.0	64.6	(45.6)	750
24c	octanoyl	$C_{17}H_{26}N_3O_8P \cdot 0.5H_2O$	AC	220–222	C, MeOH	23	235 (7900), 265 (9000)	6.3	50.7	76.0 (22.7)	(50.7)	500
24d	palmitoyl	$C_{25}H_{42}N_3O_8P$	AA	235–240	C, MeOH	8	235 (9400), 264 (11 000)	27.4	116.4	>441.1	(43.0)	500
	3',5'-di-*O*-palmitoyl-anhydro-*ara*-C HCl	$C_{41}H_{71}N_3O_6 \cdot HCl$	AC	198–199	A, EtOH	68	236 (10 000), 264 (11 500)	59.0	151.3	>528.2 (0)	(22.5)	750
	anhydro-*ara*-C HCl								42.3	76.9 (32.5)	(69.2)	3000

[a] All compounds analyzed correctly for C, H, and N except as noted. [b] AA, acid anhydride; AC, acid chloride. [c] A, trituration with ether followed by crystallization from the indicated solvent; B, trituration with ether followed by chromatography on silicic acid using 5–15% methanol in chloroform and crystallization from the indicated solvent; C, trituration with ether followed by chromatography on an anion-exchange resin (formate) using aqueous methanol and crystallization from the indicated solvent. DME, 1,2-dimethoxyethane. [d] See Experimental Section for methodology. [e] H: calcd, 4.94; found, 4.42. [f] See ref 3 and 4.

confirmed by NMR and UV spectroscopy. The NMR spectra of **24** showed the presence of an acyl group, and this function was assigned to the $C_{3'}$ position since the signal due to the $C_{3'}$-H was shifted downfield by roughly 0.9 ppm relative to that in anhydro-*ara*-C. All of the 3′-*O*-acyl derivatives **24** showed a UV spectral pattern typical of anhydro-*ara*-C itself.[20]

Anti-L1210 Activity. The various compounds described in this paper have been examined for antitumor activity against L1210 leukemia in BDF_1 mice following the general protocols outlined in the Experimental Section. From Table II it can be seen that, in general, the short-chain derivatives show modest activity at a standard dose of 100 mg/kg, ip. The long-chain diesters (**4c**, **7c**, and **12**) which have a similar chain length as the palmitoyl group exhibited a high activity. The bis[11-(butylthio)undecanoyl] ester **12**, for example, showed comparable activity (>624% ILS) to that of the dipalmitoyl ester (>528% ILS) at the same dose, ip. However, these compounds showed very modest activity via the oral route, as in the case of the dipalmitoyl ester. The long-chain diesters (**8b**, **8c**, and **13**) with an ester linkage in the side chains showed relatively low activity. The low activity of compounds **8b** and **13** may be due to the enzymatic cleavage of the side chains, which liberates the shorter-chain diesters at an early stage of absorption. The substituted dibenzoyl derivatives **14** were moderately active both ip (100 mg/kg) and po (200 mg/kg). The bis(*o*-chlorobenzoyl) (**14e**) and bis(*m*-chlorobenzoyl) (**14f**) derivatives showed significant toxicity at 100 mg/kg, ip, while this was not significant in the bis(*p*-chlorobenzoyl) isomer (**14g**). The bis(anhydro-*ara*-C 5′-)suberate derivative **22** exhibited marked toxicity at 100 mg/kg, ip, though this compound produced a 41% ILS at 200 mg/kg, po. The 3′-*O*-acyl derivatives of anhydro-*ara*-C 5′-phosphate, **24**, administered po generally showed moderate activity (43.0–50.7% ILS) at 200 mg/kg. Thus, replacement of the 5′-acyl group of 3′,5′-di-*O*-acyl-anhydro-*ara*-C, which shows only minimal activity via the oral route,[5,7] by a hydrophilic phosphoryl group results in an enhancement of the po activity. As in the case of the aliphatic acid diesters, the activity of **24** administered ip was enhanced with an increase in the chain length and the 3′-*O*-palmitoyl ester **24d** showed high activity (>441.1% ILS) at 100 mg/kg.

A number of 3′,5′-diesters of anhydro-*ara*-C with simple saturated and unsaturated aliphatic acyl groups have been examined for activity against L1210 leukemia in mice by Moffatt and co-workers.[5] They have shown an increasing tendency of the anti-L1210 activity and a decreasing tendency of the toxicity in mice as the chain of the acyl groups lengthens. Various 5′-*O*-acyl-*ara*-C compounds have also been examined for L1210 leukemia in mice by Wechter et al., and some positive correlations can be drawn between the antitumor activity of the compounds, their solubility in water, and their rate of hydrolysis by plasma esterases.[8b] As yet, we have not confirmed the solubility–activity relationship. However, it seems likely that the same relationship holds for our modified diesters.

Experimental Section

Proton magnetic resonance (NMR) spectra were obtained using a Hitachi Perkin-Elmer R-20A spectrometer. Spectra are recorded in parts per million downfield of an internal standard of tetramethylsilane. UV spectra were obtained on a Hitachi EPS-3T spectrometer. Infrared spectra were measured with a Shimadzu IR-27G spectrometer. Column chromatography was performed using Merck silica gel (0.05–0.20 mm particle size).

Materials. Boron trifluoride etherate was purified by distillation prior to use. Acetonitrile was dried over magnesium sulfate and distilled after being refluxed with phosphorus pentoxide. 3-(Ethoxycarbonyl)propionic acid,[21a] 3-[(heptyloxy)carbonyl]propionic acid,[21b] 15-(ethoxycarbonyl)pentadecanoic acid,[21c] and 11-(butylthio)undecanoic acid[22] were prepared by methods described in the literature.

Preparation of Carboxylic Acids. (a) 3-(Dodecyloxy)propionic Acid. β-Propiolactone (22 g, 0.31 mol) was added dropwise with stirring to lauryl alcohol (300 g, 1.6 mol) over a period of 20 min. The reaction mixture was warmed to 65 °C and the mixture was stirred for 16 h. The excess lauryl alcohol was distilled rapidly in vacuo. Distillation of the residue gave 23.7 g (30%) of 3-(dodecyloxy)propionic acid, bp 148–149 °C (0.15 mm), which crystallized spontaneously. An analytical sample from hexane: mp 55–56 °C; NMR ($CDCl_3$) 0.88 (t, 3, CH_3), 1.27 (s, 20, CH_2's), 2.62 (t, 2, $COCH_2$), 3.45 (t, 2, $C_{11}H_{23}CH_2O$), 3.70 (t, 2, $COCH_2CH_2O$), 10.80 ppm (br s, 1, CO_2H).

(b) 11-Phthalimidoundecanoic Acid. A mixture of 11-aminoundecanoic acid (2.0 g, 10 mmol) and phthalic anhydride (1.48 g, 10 mmol) was heated at 140–150 °C for 1 h. After cooling the mixture, the product was recrystallized from ethanol to give 2.0 g (61%) of 11-phthalimidoundecanoic acid, mp 92–93 °C.

(c) 11-(Benzoylamino)undecanoic Acid. 11-Aminoundecanoic acid (15 g, 74.5 mmol) was dissolved in hot 0.2 M sodium hydroxide (375 mL). After cooling the mixture, benzoyl chloride (9.6 mL, 82 mmol) and sodium bicarbonate (6.3 g, 74.5 mmol) were simultaneously added portionwise with vigorous stirring at 25–30 °C. The mixture was stirred at room temperature for 2 h and adjusted to pH 3 with dilute hydrochloric acid. The resulting crystals were collected by filtration, washed with water, and dried ($MgSO_4$). This material was chromatographed on a column of silicic acid using 10% methanol in chloroform. The pure product fractions were evaporated, leaving 10.5 g (46%) of 11-(benzoylamino)undecanoic acid, mp 90–92 °C.

Preparation of Acid Chlorides and Acid Anhydrides. (a) 4,5-Dihydro-2-phenyl-6*H*-1,3-oxazin-6-one Hydrochloride (17, R′ = C_6H_5, *n* = 2). Benzoyl-β-alanine (15 g, 77.5 mmol) was added portionwise with stirring to thionyl chloride (100 mL) at −5 °C. The resulting clear solution was stirred at −5 °C for 30 min and the excess thionyl chloride was then evaporated to dryness at room temperature in vacuo. The crystalline residue was washed with benzene and dried at room temperature in vacuo, giving 15 g of crude **17** (R′ = C_6H_5, *n* = 2): mp 93–95 °C dec; IR ν_{max} (Nujol) 2450 (NH^+), 1828 (C=O), 1655 cm^{-1} (C=N). This material was used in the next step without further purification.

(b) 3-(Dodecyloxy)propionic Anhydride [2a, R = $C_{12}H_{25}O(CH_2)_2$]. The above 3-(dodecyloxy)propionic acid (29 g, 0.11 mol) was added with stirring to thionyl chloride (20 g, 0.17 mol), and the mixture was heated at 70 °C for 1 h. Excess thionyl chloride was removed by evaporation in vacuo and distillation of the residue gave 25.7 g (83%) of 3-(dodecyloxy)propionyl chloride: bp 130 °C (0.5 mm); IR ν_{max} (film) 1800 cm^{-1}. The acid chloride (20.8 g, 75 mmol) was added dropwise to a stirred suspension of sodium 3-(dodecyloxy)propionate (21 g, 75 mmol) in benzene (300 mL). The mixture was stirred at room temperature for 16 h and centrifuged to remove sodium chloride. The supernatant was evaporated in vacuo and the residue was crystallized from acetone, giving 22 g (59%) of **2a** [R = $C_{12}H_{25}O(CH_2)_2$], mp 52–54 °C.

(c) 11-(Butyryloxy)undecanoic Anhydride [2a, R = $C_3H_7CO_2(CH_2)_{10}$]. A mixture of 11-bromoundecanoic acid (44 g, 0.17 mol) and sodium butyrate (84 g, 0.76 mol) in butyric acid (158 g) was heated at 120–130 °C for 12 h. Most of the butyric acid was removed by distillation in vacuo, and the residue was partitioned between ether and water. The organic phase was dried ($MgSO_4$) and evaporated. Distillation of the residue gave 16.6 g (37%) of 11-(butyryloxy)undecanoic acid: bp 183–186 °C (0.7 mm); IR ν_{max} (film) 1737, 1710 cm^{-1}. To a solution of the acid (14.5 g, 53 mmol) in tetrahydrofuran (100 mL) was added with stirring dicyclohexylcarbodiimide (5.5 g, 26.5 mmol) at −10 °C. The mixture was then stirred at 5 °C for 16 h and filtered. The filtrate was evaporated and the residue was crystallized from pentane, giving 9.6 g (68%) of **2a** [R = $C_3H_7CO_2(CH_2)_{10}$], mp 41–42 °C.

General Procedure for the Preparation of 2,2′-Anhydro-1-(3′,5′-di-*O*-acyl-β-D-arabinofuranosyl)cytosine Salts 4–16. (a) 2,2′-Anhydro-1-[3′,5′-bis-*O*-(3-ethoxypropionyl)-β-D-arabinofuranosyl]cytosine Hydrochloride (4a, X = Cl).

By Acid Anhydride Method. Purification Method B. 3-Ethoxypropionyl chloride (13.5 g, 98.9 mmol) was added dropwise to a stirred suspension of sodium 3-ethoxypropionate (13.8 g, 98.9 mmol) in tetrahydrofuran (70 mL). The mixture was stirred at room temperature for 48 h and filtered to remove sodium chloride. Evaporation of the filtrate left 20 g of crude 3-ethoxypropionic anhydride [**2a**, R = $C_2H_5O(CH_2)_2$]: IR ν_{max} (film) 1823, 1753 cm^{-1}. The crude **2a** [R = $C_2H_5O(CH_2)_2$, 13.5 g, 61.9 mmol] in acetonitrile (20 mL) was added dropwise to a stirred refluxing solution of cytidine (5 g, 20.6 mmol) and boron trifluoride etherate (7.8 mL, 61.9 mmol) in acetonitrile (150 mL) at such a rate (ca. 5 min) as to maintain refluxing. After the addition was completed, the reaction mixture was maintained at the reflux temperature for an additional 5 min. After cooling, the mixture was evaporated in vacuo and the residue was triturated with ether. The insoluble residue (11 g) was chromatographed on a column of silicic acid (180 g) using chloroform–methanol (19:1). The pure product fractions were evaporated, leaving 5.0 g of TLC- and NMR-homogeneous **4a** (X = BF_4) that could not be obtained in crystalline form. A portion of this material (3.5 g) was dissolved in 50% aqueous methanol (200 mL) and passed through a column of Amberlite IRA-400 (Cl^-, 70 mL). The column was washed with water, and the combined eluate and washings were evaporated in vacuo. The crystalline residue was recrystallized from 1,2-dimethoxyethane, giving 2.0 g (21%) of **4a** (X = Cl): mp 168–169 °C; NMR (Me_2SO-d_6) 1.10 (t, 6, CH_3), 2.2–2.8 (m, 4, $COCH_2CH_2$), 3.2–3.8 (m, 8, CH_2OCH_2), 4.0–4.3 (m, 2, $C_{5'}$-H_2), 4.5–4.8 (m, 1, $C_{4'}$-H), 5.4–5.5 (d, 1, $J_{3',4'}$ = 2 Hz, $C_{3'}$-H), 5.74 (d, 1, $J_{1',2'}$ = 6 Hz, $C_{2'}$-H), 6.68 (d, 1, $C_{1'}$-H), 6.85 (d, 1, $J_{5,6}$ = 7.5 Hz, C_5-H), 8.42 (d, 1, C_6-H), 9.6, 10.3 ppm (br s, 1, NH).

(b) 2,2′-Anhydro-1-[3′,5′-bis-*O*-[11-(benzoylamino)undecanoyl]-β-D-arabinofuranosyl]cytosine Hydrotetrafluoroborate (5c, X = BF_4). By Acid Chloride Method. Purification Method B. The above 11-(benzoylamino)undecanoic acid (10.5 g, 34 mmol) was treated with thionyl chloride (100 mL) as described for the preparation of **17** (R′ = C_6H_5, *n* = 2), giving 11.5 g of the crude azlactone hydrochloride (**17**, R′ = C_6H_5, *n* = 10) as a syrup: IR ν_{max} (film) 2250 (NH^+), 1800 (C=O), 1660 cm^{-1} (C=N). This material was added dropwise to a stirred refluxing solution of cytidine (2.3 g, 9.3 mmol) and boron trifluoride etherate (3.5 mL, 28 mmol) in acetonitrile (70 mL) over a period of 5 min. The mixture was kept at the reflux temperature for 90 min and then concentrated to dryness in vacuo. The residue was triturated with ether and purified by column chromatography on silica gel (360 g) using 15% methanol in chloroform, giving 2.6 g (32%) of **5c** (X = BF_4), mp 96–98 °C.

(c) **2,2′-Anhydro-1-[3′,5′-bis-*O*-(*p*-fluorobenzoyl)-β-D-arabinofuranosyl]cytosine Hydrochloride (14h, X = Cl). By Acid Chloride Method. Purification Method A.** *p*-Fluorobenzoyl chloride (5.0 g, 30 mmol) was added dropwise to a stirred refluxing solution of cytidine (2.6 g, 11 mmol) and boron trifluoride etherate (4 mL, 30 mmol) in acetonitrile (80 mL) over a period of 5 min. The mixture was kept at the reflux temperature for 45 min and evaporated in vacuo. The residue was triturated with ether and crystallized from ethanol, giving 4.0 g (70%) of **14h** (X = BF_4), mp 252–254 °C dec. This material was converted to the hydrochloride as described for **4a** using 50% aqueous acetone as the solvent. Crystallization from ethanol gave 3.1 g (58%) of **14h** (X = Cl), mp 214–218 °C dec.

N^4-[(Benzyloxy)carbonyl]-2′,3′-*O*-isopropylidenecytidine (18). Triethyl orthoformate (300 mL) was added dropwise to a stirred solution of *p*-toluenesulfonic acid monohydrate (40 g, 0.21 mol) in acetone (500 mL) at 0 °C. Cytidine (50 g, 0.21 mol) was then added portionwise and the mixture was stirred at 5 °C for 16 h. The mixture was neutralized with 1 N methanolic sodium methoxide (210 mL) and evaporated. The residue was dissolved in chloroform (500 mL) and filtered. The filtrate was evaporated to dryness and the residue was coevaporated twice with pyridine. The final residue was dissolved in pyridine (500 mL) and benzyloxycarbonyl chloride (87.3 g, 0.51 mol) was added dropwise at 0 °C. The mixture was then stirred at room temperature for 16 h. A further 17.5 g of benzyloxycarbonyl chloride was added at 0 °C and the mixture was stirred at room temperature for an additional 30 min. The solvent was evaporated. The residue was dissolved in chloroform (500 mL), washed with aqueous sodium bicarbonate and water, and dried ($MgSO_4$). The solvent was evaporated and the residue was crystallized from ether. Recrystallization from ethanol gave 24.3 g of **18**, mp 135–136 °C. The mother liquors from crystallization and recrystallization of **18** were combined and evaporated, leaving 65 g of a syrup. This material was shown by NMR to be a mixture of **18** and N^4,5′-*O*-[bis(benzyloxy)carbonyl]-2′,3′-*O*-isopropylidenecytidine. The mixture was dissolved in ethanol (300 mL) and treated with 1 N sodium hydroxide (177 mL) at room temperature for 2 h. The mixture was neutralized with 1 N acetic acid (177 mL) and evaporated to dryness. The residue was dissolved in chloroform (300 mL), washed with aqueous sodium bicarbonate and water, dried ($MgSO_4$), and evaporated. Crystallization of the residue from ethanol gave a further 19.5 g (total yield 43.8 g, 61%) of **18**: NMR (Me_2SO-d_6) 1.30, 1.50 (s, 3, CMe_2), 3.5–3.9 (m, 3, $C_{5'}$-H_2, OH), 4.1–4.4 (m, 1, $C_{4'}$-H), 4.73 (d, 1, $J_{2',3'}$ = 6.5 Hz, $C_{3'}$-H), 4.92 (d, 1, $C_{2'}$-H), 5.21 (s, 2, ArCH_2), 5.87 (br s, 1, $C_{1'}$-H), 7.05 (d, 1, $J_{5,6}$ = 7.5 Hz, C_5-H), 7.40 (s, 5, ArH), 8.25 (d, 1, C_6-H), 10.8 ppm (br s, 1, NH); UV λ_{max} (MeOH) 242 nm (ε 13400), 293 (5800). Anal. ($C_{20}H_{23}N_3O_7$) C, H, N; C: calcd, 57.55; found, 56.98.

Bis[N^4-[(benzyloxy)carbonyl]-2′,3′-*O*-isopropylidenecytidine 5′-]suberate (19). Suberoyl chloride (9.2 g, 0.17 mol) was added dropwise to a solution of **18** (36.4 g, 87.2 mmol) in pyridine (655 mL) at −5 °C. The mixture was then stirred at room temperature for 16 h and evaporated in vacuo. The residue was dissolved in chloroform (300 mL), washed with aqueous sodium bicarbonate and water, dried ($MgSO_4$), and evaporated. The residue was chromatographed on a column of silicic acid (300 g) using chloroform–methanol (9:1) to give 19 g (46%) of **19**: mp 77–79 °C; NMR ($CDCl_3$) 1.1–1.8 (m, 8, CH_2's), 1.33, 1.57 (s, 6, CMe_2), 2.0–2.6 (m, 4, $COCH_2$), 4.38 (br s, 6, $C_{5'}$-H_2, $C_{4'}$-H), 4.7–5.0 (m, 2, $C_{3'}$-H), 5.08 (d, 2, $J_{2',3'}$ = 6.5 Hz, $C_{2'}$-H), 5.20 (s, 4, ArCH_2), 5.70 (br s, 2, $C_{1'}$-H), 7.20 (d, 2, $J_{5,6}$ = 7.5 Hz, C_5-H), 7.37 (s, 10, ArH), 7.78 (d, 2, C_6-H). Anal. ($C_{48}H_{56}N_6O_{16}$) C, H, N.

Bis[N^4-[(benzyloxy)carbonyl]cytidine 5′-]suberate (20). A solution of **19** (16.4 g, 16.9 mmol) in 50% formic acid (400 mL) was kept at room temperature for 22 h. The mixture was evaporated below 40 °C in vacuo. The residue was dissolved in chloroform and washed with water. At this point, a white crystalline product precipitated from the organic layer. The crystals were collected by filtration and recrystallized twice from ethanol, giving 5.7 g (37%) of **20**: mp 112–113 °C; NMR (Me_2SO-d_6) 1.1–1.8 (m, 8, CH_2's), 2.2–2.5 (m, 4, $COCH_2$), 3.8–4.5 (m, 10, $C_{5'}$-H_2, $C_{4'}$-, $C_{3'}$-, $C_{2'}$-H), 5.0–6.0 (br s, 4, OH), 5.20 (s, 4, ArCH_2), 5.80 (s, 2, $C_{1'}$-H), 7.07 (d, 2, $J_{5,6}$ = 8 Hz, C_5-H), 7.40 (s, 10, ArH), 8.17 ppm (d, 2, C_6-H). Anal. ($C_{42}H_{48}N_6O_{16}$·H_2O) C, H, N.

Bis(cytidine 5′-)suberate (21). A partial solution of **20** (5.7 g, 6.4 mmol) in methanol (600 mL) was shaken in an atmosphere of hydrogen in the presence of 5% palladium on carbon catalyst (2 g) for 1.5 h. After removal of the catalyst from the solution, the solvent was evaporated, leaving 3.3 g (83%) of **21** as a foam: NMR (Me_2SO-d_6) 1.1–1.8 (m, 8, CH_2's), 2.2–2.5 (m, 4, $COCH_2$), 3.9–4.5 (m, 10, $C_{5'}$-, $C_{4'}$-, $C_{3'}$-, $C_{2'}$-H), 4.5–5.5 (br s, 4, OH), 5.80 (s, 2, $C_{1'}$-H), 5.84 (d, 2, $J_{5,6}$ = 7.5 Hz, C_5-H), 7.30 (br s, 4, NH_2), 7.62 ppm (d, 2, C_6-H).

Bis[2,2′-anhydro-1-(3′-*O*-pivaloyl-β-D-arabinofuranosyl)cytosine 5′-]suberate Dihydrochloride (22). Pivaloyl chloride (3.4 g, 28.2 mmol) was added dropwise to a stirred refluxing solution of **21** (3.2 g, 4.7 mmol) and boron trifluoride etherate (4 g, 28.2 mmol) in acetonitrile (150 mL) over a period of 5 min. The mixture was kept at the reflux temperature for an additional 10 min and evaporated to dryness in vacuo. The residue was triturated with ether, dissolved in methanol (400 mL), and passed through a column of Amberlite IRA-400 (Cl^-, 200 mL). The eluate was evaporated to dryness and the residue was crystallized from 2-propanol. Recrystallization from water gave 0.4 g (10%) of **22**: mp 215–218 °C dec; NMR (Me_2SO-d_6) 0.9–1.8 (m, 26, CMe_3, CH_2's), 1.9–2.3 (m, 4, $COCH_2$), 4.0–4.3 (m, 4, $C_{5'}$-H_2), 4.5–4.8 (m, 2, $C_{4'}$-H), 5.40 (d, 2, $J_{3',4'}$ = 2 Hz, $C_{3'}$-H), 5.75 (d, 2, $J_{1',2'}$ = 5.5 Hz, $C_{2'}$-H), 6.72 (d, 2, $C_{1'}$-H), 6.83 (d, $J_{5,6}$ = 7.5 Hz, C_5-H), 8.42 (d, 2, C_6-H), 9.60, 10.13 ppm (br s, 2, NH); UV λ_{max} (MeOH) 235 nm (ε 20900), 264 (23800). Anal. ($C_{36}H_{50}Cl_2N_6O_{12}$·$2H_2O$) C, H, N.

Reactions of 5′-Cytidylic Acid with Acid Anhydrides or Acid Chloride. A Typical Example. 2,2′-Anhydro-1-(3′-*O*-valeryl-β-D-arabinofuranosyl)cytosine 5′-Phosphate (24b).

A solution of valeric anhydride (5.2 g, 27.8 mmol) in acetonitrile (20 mL) was added dropwise to a stirred refluxing solution of 5′-cytidylic acid (3 g, 9.3 mmol) and boron trifluoride etherate (3.6 mL, 27.8 mmol) over a period of 7 min. The mixture was kept at the reflux temperature for an additional 10 min. The solvent was evaporated in vacuo and the residue was triturated with ether (3 × 100 mL). A further 100 mL of ether was added to the final residue and the mixture was stirred at room temperature for 16 h. The product (5.9 g) was collected by filtration, dissolved in 30% aqueous methanol (500 mL), and passed through a column of Diaion SA-11B (formate, 60 mL). The column was washed with the same solvent (150 mL) and the combined eluate and washings were evaporated to dryness in vacuo. The residue was triturated with ethanol (200 mL) and coevaporated three times with methanol, giving 1.1 g (31%) of **24b**: mp ~225 °C dec; NMR (D_2O) 0.90 (t, 3, CH_3), 1.1–1.9 (m, 4, CH_2's), 2.3–2.7 (m, 2, $COCH_2$), 3.8–4.1 (m, 2, C_5-H_2), 4.07 (m, 1, C_4-H), 5.60 (br s, 1, C_3-H), 5.78 (d, 1, $J_{1',2'}$ = 6 Hz, $C_{2'}$-H), 6.62 (d, 1, $J_{5,6}$ = 7.5 Hz, C_5-H), 6.69 (d, 1, $C_{1'}$-H), 8.11 ppm (d, 1, C_6-H); UV λ_{max} (MeOH) 235 nm (ε 7000), 264 (7700).

Biological Test Systems. BDF_1 mice were inoculated intraperitoneally with 1×10^5 L1210 cells. The test substance was either dissolved or suspended in 0.9% aqueous sodium chloride containing 0.1% NIKKOL[23] HCO-60 (Nikko-Chemicals Co., Ltd.) and was administered intraperitoneally (ip) and/or orally (po). Treatment was started 24 h after the leukemic cell inoculation and continued daily for 5 days. The daily doses employed in ip administration were 100 and 25 mg/kg and in certain cases a lower dose, 6.25 mg/kg, was also examined. In the case of po administration, the doses were 200 and 100 mg/kg. Groups of six to ten mice were used. Antitumor activity was calculated by comparing the mean survival time of the treated animals (T) to that of the control animals (C), i.e., the percentage increase in life span (ILS); (T/C − 1) × 100 (%). The observation periods were 30 (po) and 60 (ip) days. Maximum tolerated dose (MTD) for mice was evaluated by a single intraperitoneal injection of each test substance to three mice (female ICR mice weighing 20–21 g). The observation period was 3 weeks. Test data for the leukemic mice are listed in Table II and data for anhydro-*ara*-C hydrochloride and 3′,5′-di-*O*-palmitoyl-anhydro-*ara*-C hydrochloride are included for comparison.

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References and Notes

(1) M. C. Wang, R. A. Sharma, and A. Bloch, *Cancer Res.*, 33, 1265 (1973).
(2) (a) A. Hoshi, F. Kanzawa, K. Kuretani, M. Saneyoshi, and Y. Arai, *Gann*, **62**, 145 (1971); (b) D. T. Gish, G. L. Neil, and W. J. Wechter, *J. Med. Chem.*, 14, 882 (1971); (c) A. Hoshi, F. Kanzawa, and K. Kuretani, *Gann*, **63**, 353 (1972); (d) J. M. Venditti, M. C. Baratta, N. H. Greenberg, B. J. Abbott, and I. Kline, *Cancer Chemother. Rep.*, **56**, 483 (1972).
(3) T. Kanai and M. Ichino, *Tetrahedron Lett.*, 1965 (1971).
(4) A. Hoshi, F. Kanzawa, K. Kuretani, T. Kanai, K. Kikugawa, and M. Ichino, *Gann*, **64**, 519 (1973).
(5) E. K. Hamamura, M. Prystasz, J. P. H. Verheyden, J. G. Moffatt, K. Yamaguchi, N. Uchida, K. Sato, A. Nomura, O. Shiratori, S. Takase, and K. Katagiri, *J. Med. Chem.*, **19**, 663 (1976).
(6) K. Kondo and I. Inoue, *J. Org. Chem.*, **42**, 2809 (1977).
(7) Unpublished work by Y. Arai, K. Kondo, and I. Inoue.
(8) (a) G. D. Gray, F. R. Nichol, M. M. Mickelson, G. W. Camiener, D. T. Gish, R. C. Kelly, W. J. Wechter, T. E. Moxley, and G. L. Neil, *Biochem. Pharmacol.*, **21**, 465 (1972); (b) W. J. Wechter, M. A. Johnson, C. M. Hall, D. T. Warner, A. E. Berger, A. H. Wenzel, D. T. Gish, and G. L. Neil, *J. Med. Chem.*, 18, 339 (1975).
(9) D. H. W. Ho and G. L. Neil, *Cancer Res.*, **37**, 1640 (1977).
(10) T. L. Gresham, J. E. Jansen, F. W. Shaver, J. T. Gregory, and W. L. Beears, *J. Am. Chem. Soc.*, **70**, 1004 (1948).
(11) J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acid", Wiley, New York, N.Y., 1961, p 885.
(12) See, e.g., R. G. Jones, *J. Am. Chem. Soc.*, **69**, 2350 (1947).
(13) See, e.g., A. F. Beecham, *J. Am. Chem. Soc.*, **79**, 3257 (1957).
(14) Zinner et al. treated benzoyl-β-alanine with phosphorus pentachloride in acetyl chloride and isolated a crystalline product. The structure of this compound was assigned benzoyl-β-alanyl chloride [H. Zinner and G. Brossmann, *J. Prakt. Chem.*, **5** (4), 91 (1957)]. However, no spectral data were given in the literature.
(15) S. Satsumabayashi, S. Motoki, and H. Nakano, *J. Org. Chem.*, 41, 156 (1976).
(16) C. Rüchardt, O. Krätz, and S. Eichler, *Chem. Ber.*, **102**, 3299 (1969).
(17) E. K. Hamamura, M. Prystasz, J. P. H. Verheyden, J. G. Moffatt, K. Yamaguchi, N. Uchida, K. Sato, A. Nomura, O. Shiratori, S. Takase, and K. Katagiri, *J. Med. Chem.*, **19**, 654 (1976).
(18) See, e.g., J. March, "Advanced Organic Chemistry: Reactions, Mechanisms, and Structure", McGraw-Hill, New York, N.Y., 1968, p 345.
(19) See, e.g., J. O. Edwards and R. G. Pearson, *J. Am. Chem. Soc.*, **84**, 16 (1962).
(20) E. R. Walwick, W. K. Roberts, and C. A. Dekker, *Proc. Chem. Soc.*, 84, (1959).
(21) (a) W. V. Miller and H. Hofer, *Chem. Ber.*, **28**, 2431 (1895); (b) J. A. Southern and C. D. Riddle, *Furman Univ. Bull., Furman Studies*, **8**, 6 (1961); *Chem. Abstr.*, **55**, 17131e (1961); (c) R. Goto, A. Ishizawa, and M. Yamamura, *Nippon Kagaku Zasshi*, 88, 678 (1967); *Chem. Abstr.*, **68**, 86865z (1968).
(22) N. H. Koenig and D. Swern, *J. Am. Chem. Soc.*, **79**, 4235 (1957).
(23) Hydrogenated castor oil polyethylene glycol ether.